BC Environment

A REVIEW OF THE ENVIRONMENTAL IMPACT AND TOXIC EFFECTS OF 2-MBT

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SUMMARY

This paper summarizes the physical, chemical, environmental, and toxicological properties of 2-mercaptobenzothiazole (2-MBT). 2-MBT is used in the production of the antisapstain compound 2-(thiocyanomethylthio)benzothiazole (TCMTB), and is one of its breakdown products. 2-MBT is also used in the rubber industry as an accelerant in the vulcanizing process, and as an antioxidant, an anticorrosion agent, and a textile preservative.

2-MBT is stable to environmental hydrolysis, but undergoes photolysis in pure water and in water containing dissolved humic acids. It undergoes limited biodegradation, and does not support growth of soil microbes. 2-MBT may undergo biologically-mediated methylation to form 2-methylthio(benzothiazole). Its greatest threat to animal species results from aquatic exposure and accumulation of high density rubber dust in certain areas, such as those close to rubber manufacturing plants. The compound is not expected to bioconcentrate.

In rats, 2-MBT is readily absorbed into the bloodstream. However, only a small portion remains associated with erythrocytes 96 hours after dosing. It is not retained in other tissues. It is metabolized to several conjugated compounds, and excreted mainly in the urine.

According to the scales used in this report, 2-MBT is moderately toxic orally, and slightly toxic dermally to laboratory animals. Sensitization studies give contradictory results, but numerous reports of human sensitization to the chemical are available. Toxicity ratings for fish range from slightly toxic to very toxic, depending on species. In terms of cell count, 2-MBT is very toxic to at least one algal species.

The carcinogenic potential of 2-MBT has not been conclusively established. Several mutagenic tests have given negative results, but the chemical has been shown to be clastogenic and genotoxic. Reproductive studies have also given contradictory results. Teratogenic effects have been noted in some instances, but other studies have found no evidence of teratogenicity, maternal toxicity, or fetotoxicity.

Dermal contact and inhalation are expected to be the primary routes of occupational exposure. Skin contact may result in contact dermatitis in susceptible individuals.

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1.0 INTRODUCTION

One antisapstain chemical presently used to replace chlorinated phenols in the lumber industry is 2-(thiocyanomethylthio)benzothiazole (TCMTB). TCMTB is an active component in a number of formulations, including Busan 1030 and Busan 30WB, both used in wood protection in Canada and produced by Buckman Laboratories of Canada Ltd. TCMTB has a number of other uses, including prevention of seed and soil-borne diseases, and control of microorganisms in cooling water systems, crude and refined oils, drilling fluids, and paints (1).

2-Mercaptobenzothiazole (2-MBT) is a starting compound used in the production of TCMTB. It is also an impurity in the final product, and one of its primary degradation products (1). Since 2-MBT is expected to be found in the environment wherever TCMTB is used, it is useful to examine its toxic and environmental effects in relation to wood treatment.

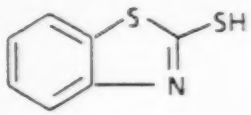
The purpose of this paper is to review the physical, environmental, and toxicological properties of 2-MBT. The toxicological and environmental effects of TCMTB have recently been reviewed by the BC Ministry of Environment (1). Several computerized databases, listed in Appendix A, were searched for information on 2-MBT, and some information was supplied by manufacturers of the chemical.

2.0 HISTORICAL BACKGROUND AND USES

2-MBT was originally used in the late 1940's as an accelerant in the vulcanizing process in rubber manufacture. Since that time, it has found widespread use as an antioxidant in the rubber industry, as an anticorrosion agent in metal processing and related applications (3), as a preservative in textile manufacturing (20), and as a fungicide and bacteriostatic agent (3). More recently, 2-MBT has been used in the synthesis of the antisapstain chemical TCMTB (1). The main North American manufacturers of 2-MBT are BF Goodrich, Goodyear Tire, Uniroyal Chemical, and Monsanto Chemical (21).

3.0 PHYSICAL AND CHEMICAL PROPERTIES

The structural formula of 2-MBT is shown below:



(CAS Registry No.: 149-30-4)

Technical 2-MBT is a yellowish to tan crystalline powder with a distinct, disagreeable odour. The solubility of 2-MBT in water under various conditions has been measured, as follows: 332 mg/L, pH unspecified (7); 51 mg/L at pH 5, 118 mg/L at pH 7, and 900 mg/L at pH 9 (7); 120 mg/L at 24°C (73), 54 mg/L at 5°C (73), and 100-120 mg/L at 20°C (74). Solubility has also been measured in other solvents, including ethyl alcohol (20 g/L), acetone (100 g/L) (4), benzene, and chloroform (5).

2-MBT has a specific gravity of 1.42-1.5 at 25°C, a vapour pressure of 24 mm Hg at 20°C, and a melting point of approximately 180°C. Decomposition occurs above 260°C. The log octanol-water partition coefficient has been estimated at 1.61 (6), and measured at 2.24 (7). Further research on 2-MBT found log Kow's of 2.41 at 24°C, 1.43 at pH 6.5 and 23°C, 1.38 at pH 7.5 and 23°C, and 0.95 at pH 8.5 and 24°C (73).

2-MBT is manufactured by reacting aniline with equimolar quantities of sulfur and carbon disulfide at elevated temperature and pressure (22). Indirect production of the chemical can result during the vulcanization process from the breakdown of 2-MBT-derived accelerators (23), and from the production, photolysis, hydrolysis, and decomposition of TCMTB (1).

2-MBT is a weak acid and forms salts in basic solutions with a variety of metals. Its sodium salt is a commercially available product. In acid solution, it can be reduced in the presence of iron to benzothiazole (BT); on contact with acid or acid fumes it emits highly toxic fumes of sulfur compounds (e.g. hydrogen sulfide, carbon disulfide) (59). In the presence of ozone and potassium iodide, 2-MBT dimerizes to 2-mercaptobenzothiazole disulfide (14).

Combustion of 2-MBT liberates carbon monoxide, carbon dioxide, nitrogen oxides, and sulfur dioxide (4). No quantitative thermal decomposition studies were located. Decomposition in a sealed system at 370°C produced both hydrogen sulfide and carbon disulfide (65).

4.0 ENVIRONMENTAL IMPACT

4.1 Environmental Sources

2-MBT is unlikely a naturally occurring compound. Sources in the environment could include chemical spills, unintentional and intentional discharges from commercial facilities, and discharges from products containing 2-MBT, such as tires and other rubber products. Additional discharges occur from chemicals that decompose to give 2-MBT, such as other rubber accelerants and TCMTB. In 1985, the United States Environmental Protection Agency (USEPA) (5) estimated that 1,000,000 pounds (450,000 kg) of 2-MBT are released to the environment annually from the manufacturing, processing, use, and disposal of 2-MBT. It was also estimated that a tire plant producing 25,000 steel belted radial tires per day would release 158 lbs (72 kg) of 2-MBT annually. Estimates of 2-MBT from TCMTB sources were not found in the literature, but it appears that this could be a substantial source.

4.2 Environmental Distribution

Several studies on environmental levels of 2-MBT have been done, but these focussed on concentrations near 2-MBT manufacturing sites. The USEPA (5) estimated the mass distribution of 2-MBT in air, water (including sediments), and soil to be ~0.0003, 9.9933, and 0.0084, respectively, on a relative scale. Mean concentrations of 2-MBT in 18 surface water samples, far removed from potential industrial contamination sources, were all below the detection limit of 10 ppb (24).

Two studies on 2-MBT levels at mills using TCMTB for wood treatment were located. In the first case (13), contaminated wood leachates contained 407 ppb 2-MBT, and stormwater runoff contained 1170 ppb. In the second case (57), wood leachate from freshly sprayed lumber contained less than 50 ppb 2-MBT, while yard runoff contained an average of 1155 ppb. With more than 24 hours covered storage of the lumber after treatment, 2-MBT in lumber leachate measured between less than 25 ppb and 4740 ppb. Measurements of 2-MBT in the Fraser River, BC adjacent to a rough cut storage yard were all below 25 ppb. Levels of 2-MBT in stormwater runoff are increased relative to those in lumber leachate probably because of hydrolytic and photolytic breakdown of TCMTB to 2-MBT over time.

4.3 Environmental Behaviour

In general, 2-MBT is nonvolatile and will tend to partition mainly to water rather than to soil and air (5). Moderate adsorption to soil or sediment has been observed (25), but details were not provided. Because of its moderate octanol/water partition coefficient (log Kow = 0.95-2.41), it is not expected to bioconcentrate significantly. A bioconcentration factor of 25 has been estimated (24). However, 2-MBT may be methylated in the environment (e.g. soil and sediments) to form 2-(methylthio)benzothiazole (MTBT), a chemical which has been shown to bioaccumulate (58).

4.3.1 Hydrolysis/Photolysis

Since high pH is required for hydrolysis, 2-MBT is not expected to hydrolyze under environmental conditions. It has been demonstrated that 2-MBT undergoes photolysis in pure water and water containing dissolved humic acids (26) to produce benzothiazole (BT) as the main degradation product (58). One study on the photolysis of 2-MBT found that UV-irradiation of the chemical in the presence of oxygen in a pyrex flask yielded bis-(2-benzothiazolyl) disulfide when dry benzene or toluene was used as the reaction medium (60). With solutions of 2-MBT in methanol, ethanol, or acetonitrile, bis-(2-benzothiazolyl) disulfone was obtained as the intermediate, and benzothiazole sulfate was the final reaction product.

To identify the mechanism of photolysis operative under natural conditions, sunlight photolysis of 2-MBT in dilute phosphate buffer with and without dissolved organic matter (DOM), and in natural water was carried out (73). After irradiation of 2-MBT in phosphate buffer at pH 7, 2-hydroxybenzothiazole (HOBT) (4%), benzothiazole (11%), and traces of an unidentified product were produced (73). Acidification of the aqueous phase resulted in the production of a further 18% yield of BT. Similar but slightly higher yields were obtained for irradiation in the presence of 10 mg/L DOM. In natural water (pH 7.1), irradiation of 2-MBT yielded 5% HOBT and 21% BT; acidification yielded a further 26% of BT. Traces of 2,2'-dithiobis(benzothiazole) (MBTS) (1-2%) were indicated by HPLC, but not confirmed. From these results, BT and HOBT are anticipated to be the stable products resulting from photolysis of 2-MBT in aquatic environments (73).

Further research on 2-MBT photolysis under simulated natural conditions found that the half-life of 2-MBT is on the order of one day or less for dilute solutions in surface waters and full sunlight, even in the presence of moderate concentrations of DOM (73). Direct photolysis is therefore considered a plausible route for rapid degradation of 2-MBT in well-mixed receiving waters, in the absence of high turbidity or ice cover (73).

4.3.2 Biodegradation

Some evidence of biodegradation of 2-MBT is available. 2-MBT is oxidized by mixed cultures and sludge microorganisms under aerobic conditions (27), but was found to be degradation resistant with activated sludge inoculum (28). No degradation was seen after 30 days using a Chemical Oxygen Demand (COD) method (29), and only 2 percent of maximum CO_2 evolution was found, indicating 2-MBT was essentially nondegradable under the conditions of the experiment. It has also been reported that 2-MBT at a 0.1% concentration completely retarded the growth of soil microbes (30).

2-MBT incubated in the presence of suspended sediment was converted to MTBT in low yield, probably by biologically-mediated methylation of the mercapto group (73). This route offers a plausible means by which 2-MBT can be converted into the relatively stable MTBT (73).

Anaerobic degradation of 2-MBT by sulfur bacteria in the aquatic environment has also been studied (69). The degradation rate was estimated in the presence and absence of glucose, at concentrations of 5 and 10 ug 2-MBT/mL. At both concentrations degradation was slowed by the addition of 0.1% glucose, although quantitative data were not available. After five weeks of incubation in the absence of glucose, 44% and 74% of 2-MBT was degraded in samples containing 10 and 5 ug 2-MBT/mL, respectively.

4.3.3 Biological Exposure

It appears that the greatest potential for the exposure of aquatic species, including fish, to 2-MBT is through wastewater from plants manufacturing, processing, and using 2-MBT and its derivatives. Exposure of terrestrial populations is also expected in areas accumulating a high-density of rubber dust, or in areas receiving waste rubber (21). Rubber residues on pavement and stormwater from scrap rubber yards could potentially result in runoff containing measurable levels of 2-MBT, but no studies on these sources were located. In British Columbia, additional exposure may result from the degradation of TCMTB used in wood treatment, but again no studies comparing source levels were located.

4.4 Environmental Fate Pathway

Based on the research above, and especially on one study (73), a partial pathway for 2-MBT in aquatic systems can be formulated. 2-MBT can either photolyze to BT and HOBT, or undergo biomethylation to MTBT. These stable products have been reported in aquatic systems (73). Under conditions using chlorine (e.g. drinking water or sewage disinfection), BT and MTBT may also undergo oxidation by hypochlorite to HOBT (75), and to 2-(methylsulfinyl)benzothiazole (MSiBT) and 2-(methylsulfonyl)benzothiazole (MSoBT), respectively (73). Sulfenamides of 2-MBT may also transform to 2-MBT (76), which may then be photolyzed to BT (73).

5.0 PHARMACOKINETICS

5.1 Absorption, Transport, and Distribution

Three oral and intravenous dosage studies have been done on rats. In the first study (16), groups of four F344 rats of each sex were dosed orally with a low or high dose (0.592 or 55.5 mg/kg) of carbon-14 labelled 2-MBT, then sacrificed at selected times after dosing (8, 24, 48, 72, and 96 hr). Whole blood, plasma, urine, and feces were collected and analyzed for radioactivity. 2-MBT was readily absorbed into the bloodstream, and a small portion of the radioactivity remained associated with the erythrocytes at 96 hours after dosing. A saturable process may operate at high doses, since a greater percentage of the total dose was found in whole blood and plasma at the low dose than at the high dose. Although non-blood tissues were not examined, the recovery data did not indicate retention in the body.

In a second similar study (17), groups were dosed orally with unlabelled 2-MBT for 20 days prior to single dosing with carbon-14 labelled 2-MBT. The average dose of the unlabelled compound was 0.510 mg/kg/day; the dose of the labelled compound was 0.503 mg/kg. Animals were again sacrificed at selected times (8, 24, 48, 72, and 96 hr), and whole blood, plasma, various tissues, urine, and feces were collected and analyzed for radioactivity. Results showed that a small portion of the administered radioactivity (1.20-1.69% of dose) remained associated with the erythrocytes 96 hours after dosing, and was mainly bound to membranes. Only trace amounts of radioactivity remained in other tissues at this time, with the thyroid retaining the highest (unspecified) concentration. Retention in other tissues was negligible.

In the third study (18), groups of four F344 rats of each sex were dosed intravenously with 0.602 mg/kg of carbon-14 labelled 2-MBT and sacrificed at selected times (5 min, 15 min, 1hr, 2hr, 4hr, 24hr, and 72hr). Whole blood, plasma, urine, and feces were collected and analyzed for radioactivity, and a profile of urinary metabolites was obtained. Results showed that a small portion (1.52-1.96%) of the administered radioactivity was associated with the erythrocytes 72 hours after dosing. Although non-blood tissues were not examined in this study, the recovery data did not indicate that appreciable amounts of radioactivity from ^{14}C-2-MBT were retained in tissues other than blood.

In a study on pharmacokinetics of 2-MBT in carp (*Cyprinus carpio*) (15), carbon-14 labelled 2-MBT was infused into the anterior intestine and the fish were then either fed or starved for variable periods of time. Results showed that 2-MBT was still being absorbed 24 hours after infusion, but by 72 hours absorption was complete. Accumulation of radioactivity, probably as MBT-glucuronide, occurred predominantly in the bile. When carp were starved after administration, 2-MBT was absorbed and distributed in the whole body. When the fish were fed, 2-MBT was rapidly excreted, probably mainly via the urinary system. Mechanisms of transport were not discussed.

Absorption, tissue distribution, and metabolism studies of radiolabelled 2-MBT in guinea pigs showed that the chemical was absorbable through the skin and that abrasion increased the rate of absorption (35). Ninety percent of the compound underwent conjugation with glucuronides and sulfates and was excreted in the urine 6 hours after topical application. After subcutaneous injection of 2-MBT in guinea pigs, the kidney, liver, and thyroid gland were the initial principal organs of uptake. The thyroid gland ultimately attained the highest concentration 48 hours after injection, although this concentration was not specified (35).

5.2 Metabolism and Excretion

The rat studies outlined above also examined excretion and metabolism of 2-MBT. The first study (16) showed that 72.1 to 106% of 2-MBT was excreted in the urine within 96 hours, and that 4.03 to 10.3% of the radioactivity was excreted in the feces within this time. Urinalysis revealed two major and five minor

metabolites, found in various dosed groups. None of the metabolites were identified. 2-MBT was seen only in urine from low-dose rats.

The second study (17) differentiated excretion of 2-MBT in the two sexes. For male and female rats, 90.7% and 101%, respectively, of the radioactivity administered was excreted in the urine within 96 hours of dosing. Similarly, 9.99% and 5.22% of the dose, respectively, was excreted in the feces over the same time period. Urinalysis revealed only two metabolites, the major one being a thioglucuronide conjugate of 2-MBT, and the minor one possibly being a phenol metabolite.

The third study (18) gave similar results to the first two, with 90.9 to 101% of radioactivity being excreted in the urine within 72 hours, and 3.79 to 15.1% of radioactivity being excreted in the feces. Preliminary urinalyses showed two major and two minor metabolites, none of which were identified. The parent compound was seen only in small amounts in the urine from a female rat.

In the carp study (15), feeding after 2-MBT administration resulted in most of the chemical being excreted. Radioactivity in the bile was less than 0.3% after 72 hours, and a negligible amount remained in the other tissues. It seems that bile containing radioactive metabolites was secreted into the intestine to support digestion, and that this was the main route of excretion of the chemical. After one hour of starvation, the radioactivity remaining in the intestine was 45% of the original dose, and after 72 hours, the activity in the bile was about 9%. In the other tissues, less than 0.05% of the dose was detected. Analysis of bile from carp starved for 24 hours after receiving the chemical showed that the radioactive parent was hydrolyzed by B-glucuronidase in the bile to give MBT-glucuronide. Additionally, a portion of the 2-MBT was oxidized to 2,2'-dithiobis(benzothiazole). Renal excretion appeared to be the main route of excretion in this study since radioactivity was high in the kidney and urinary bladder one hour after administration.

The urinary metabolites of [^{35}S-mercapto]2-mercaptobenzothiazole in rats dosed by intraperitoneal injection consisted of conjugates of glutathione, glucuronic acid, and inorganic sulfate (36). The authors suggested three possible metabolic pathways for 2-MBT, starting with a benzothiazole-2-glutathione metabolite. This intermediate was either reduced via benzothiazole-2-cysteine to benzothiazole-2-mercapturic acid, or via benzothiazole-2-mercaptan to either benzothiazole-2-mercaptoglucuronide or inorganic sulfate.

5.3 Placental and Milk Transfer

No studies on placental or milk transfer were located.

6.0 TOXICOLOGICAL EFFECTS

6.1 General Toxicity

There are few published studies on the toxicity of 2-MBT. Confidential information may be available from major manufacturers, but only Material Safety Data Sheets (MSDSs) were obtained for this review. Results from MSDSs and from published studies are summarized below.

6.2 Mammalian Toxicity

The following toxicity values are assumed to be for pure 2-MBT, although purity may not have been indicated in original sources. Toxicity ratings follow each oral and dermal toxicity value and reference, according to the table in Appendix B.

			Toxicity
a) Acute Oral LD50:			
	Mouse	1851 mg/kg (8)	3
	Mouse	2000 mg/kg (12)	3
	Mouse	1558 mg/kg (males) (67)	3
	Mouse	1490 mg/kg (females) (67)	3
	Mouse	3148 mg/kg (males) (67)	3
	Rat	1680 mg/kg (8)	3
	Rat	3800 mg/kg (4)	3
	Rat	500 mg/kg (10)	3
	Rat	100 mg/kg (62)	4
b) Acute Dermal LD50:	Rabbit	>7940 mg/kg (4)	2
	Rabbit	>5010 mg/kg (11)	2
c) Acute Intraperitoneal LD50:			
	Mouse	440 mg/kg (12)	
	Mouse	100 mg/kg (8)	
	Rat	300 mg/kg (8)	
d) Eye Irritation:	Rabbit	slightly irritating (4)	
e) Skin Irritation:	Rabbit	nonirritating (4)	

f) Dermal Sensitization:

2-MBT is a strong contact allergen to guinea pigs (4). However, Monsanto has reported one study in which a repeat insult patch test was done on 50 human volunteers. It was concluded in the study that 2-MBT is not a primary skin irritant, a cumulative irritant, or a sensitizing agent (4). On the other hand, numerous studies and medical reports have found that 2-MBT is one of the most common contact allergens known today (5). The general consensus appears to be that 2-MBT is a potential sensitizer for some individuals.

A study on guinea pigs found that the sensitization potential of 2-MBT appeared to be directly related to the sulfhydryl functional group in the benzothiazole structure (66).

			Toxicity
g) Inhalation LC50*:	Rat	>1270 mg/m^3 (4)	1

* assumed to be a four hour exposure period

h) Feeding Studies:

Results from four feeding studies evaluating chronic toxicity were found. They are summarized below.

The chronic toxicity of 2-MBT was evaluated by administering the chemical by gavage to groups of 10 male and 10 female rats and mice at dose levels of 0, 188, 375, 750, and 1500 mg/kg/day, 5 days per week over 13 weeks (14). Male mice showed increased mortality at 1500 mg/kg and female mice showed increased mortality at 750 and 1500 mg/kg. Average liver weights for both sexes of mice were evaluated at the highest dose, but no treatment-related histological lesions were noted. The rats did not show increased mortality, but did show reduced body weights at the three highest levels. Relative liver weights were significantly increased in both sexes of all treatment groups, but no histopathological lesions of the liver were found. A No Observed Effect Level (NOEL) was not established.

The toxicity of 2-MBT was also evaluated in B6C3F1 and B6AKF1 mice (4). Animals were initially administered the test material by gavage at a daily dose of 100 mg/kg from 7 to 28 days of age. Animals were then divided into two groups. The first group continued to receive 2-MBT by gavage, while the second group was given a single subcutaneous injection of 215 or 1000 mg/kg at 28 days of age. An increase in reticular cell sarcomas at the site of injection was observed in B6C351 mice dosed subcutaneously at 215 mg/kg; sarcomas were also seen in animals given 2-MBT daily in the diet. No significant increase in sarcoma incidence occurred in the B6AKF1 strain. Again, a NOEL was not established.

A third study in which rats received 500 mg/kg of 2-MBT daily for one week showed more severe toxic results. Effects included hepatic damage, with focal necrosis, and profound changes in cell structures, including accumulation of cytoplasmic granules, nuclear changes, and ruptured bile canaliculi with bile stasis (10).

A 20-month chronic toxicity study on mice was carried out with dose levels of 30, 120, 480, and 1920 ppm in the diet (67). Inhibition of bodyweight gain was observed in the highest dose group of males from the initial stage of treatment. Although further details were unavailable, a NOEL of 120

ppm 2-MBT, or 14.6 mg/kg/day for males and 13.52 mg/kg/day for females, was established.

6.3 Fish Toxicity

The following fish toxicity tests were done under either static (s), flowthrough (f), or unspecified (u) conditions. 2-MBT was assumed to be 100% pure in each case. Toxicity ratings follow each 96 hour LC50 toxicity value and reference, according to the table in Appendix C.

			Toxicity
a) 48 hr LC50:	Goldfish	(u) 2 ppm (9)	
	Fingerling Trout	(f) 1.14 ppm (31)	
b) 96 hr LC50:	Rainbow Trout	(s) 0.75 ppm (31)	4
	Bluegill Sunfish	(s) 1.5 ppm (32)	3
	Fingerling Trout	(f) 0.73 ppm (32)	4
	Fathead Minnow	(s) 11 ppm (32)	2
c) 192 hr LC50:	Fingerling Trout	(f) 0.67 ppm (31)	

6.4 Zooplankton Toxicity

a) 24 hr LC50:	*Daphnia magna*	(s) 7.0 ppm (33)
b) 48 hr LC50:	" "	(s) 4.1 ppm (33)
c) 192 hr LC50:	" "	(s) 0.67 ppm (33)

6.5 Algal Toxicity

			Toxicity
a) 96 hr EC50:	*Selenastrum* species		
	(cell count)	0.25 ppm (4)	4
	(cell count)	250 ppm (34)	1
	(chlorophyll)	230 ppm (34)	1

6.6 Comparative Toxicity

Since 2-MBT may be used in the manufacture of the antisapstain chemical TCMTB and is also a primary degradation product of this compound, a comparison of their relative toxicities is warranted. Table 1 lists some toxicity values for 2-MBT, TCMTB, and Busan 1030, a 30% solvent-based formulation of TCMTB. 2-MBT is approximately twice as toxic orally to laboratory rats than are either 80% TCMTB or Busan 1030. However, 2-MBT is much less toxic dermally than are the other two products. Perhaps more importantly, 2-MBT is much less toxic to salmonid species than are either TCMTB or Busan 1030. These aquatic toxicity values are crucial to the establishment of legislation regarding effluent discharges from facilities using these chemicals.

It should be noted here that although this analysis may portray 2-MBT as a generally less toxic chemical than TCMTB, other data, including those from

carcinogenicity, mutagenicity, and teratogenicity studies, should be considered in the overall assessment of each chemical compound.

7.0 CARCINOGENICITY

The carcinogenic effects of 2-MBT are unclear, although it does seem to exhibit some carcinogenic potential. Results of several studies are summarized below.

The National Toxicology Program (NTP) reported the results of carcinogenicity bioassays with 2-MBT in F344/N rats and B6C3F1 mice (14). Fifty animals of each species and sex were administered the chemical in corn oil by gavage, 5 days per week for 103 weeks. Male rats and both sexes of mice were given 375 or 750 mg/kg/day, and female rats were given 188 or 375 mg/kg/day.

The report concluded that there was some evidence of carcinogenic activity in male and female rats. Male rats showed an increased incidence of mononuclear cell leukemia and pancreatic cell adenomas at the low dose only. Adrenal gland pheochromocytomas, malignant pheochromocytomas, and preputial gland adenomas or carcinomas were more frequent in male rat treatment groups than in control groups. Female rats showed an increased incidence of adrenal gland pheochromocytomas and pituitary gland adenomas in both treatment groups.

The report further stated that there was no evidence of carcinogenicity in male mice, and equivocal evidence for carcinogenic activity in female mice on the basis of an increased incidence of hepatocellular adenomas in the low dose group. The tumour types reported are commonly observed as spontaneous lesions in this species and strain. The significance of the increased incidence seen in treated animals is uncertain due to the probable adverse effects of the excessive dose regimens and the corn oil vehicle gavage.

In another study (37), rats which were fed levels of 0, 500, 1580, or 5000 ppm of a formulation consisting of 2.4% 2-MBT and 27.6% dimethyl-dithiocarbamate for 2 years showed no increase in tumour incidence. The dietary concentrations of 2-MBT in this study were 0, 12, 37.9, and 120 ppm, but other details of the study were not available.

A third study (38) showed no increase in the incidence of tumours in two hybrid strains of mice after 18 months of administering 2-MBT. At age 7 to 28 days, mice were administered doses of 100 mg/kg by gavage, and thereafter fed a concentration of 323 ppm *ad libitum*. Again, further details were not available.

An investigation of a possible genotoxic mechanism for the carcinogenicity of 2-MBT was done by examining the covalent binding of 2-MBT to DNA from rat tissues (68). Male and female F344 rats were dosed by gavage with 375 mg ^{14}C-2-MBT/kg and killed eight hours later. DNA was extracted from the liver, adrenal glands, pituitary gland, pancreas, and bone marrow and the amount of

radioactivity associated with the DNA was determined by liquid scintillation spectroscopy. Assay results from the liver demonstrated only 0.6% of the 2-MBT radioactivity from the parent compound, while the pancreas, adrenal gland, pituitary gland, and bone marrow each exhibited less than 0.03% of this dose.

Results indicated that the overall DNA binding of 2-MBT was highest in the livers of male and female rats, with the covalent binding indices being 3.11 and 1.65, respectively. The authors concluded that 2-MBT does not significantly bind to the liver, pancreas, bone marrow, adrenal or pituitary gland DNA. They further suggested that 2-MBT does not exhibit a genotoxic mechanism for carcinogenicity.

8.0 MUTAGENICITY

Testing of 2-MBT in bacteria demonstrated no mutagenic activity, although clastogenic and genotoxic effects have been demonstrated in mammalian cells in culture (14). One study (39) found no increase in *Salmonella typhimurium his+* revertant colonies after exposure to 2-MBT. An earlier study (39) showed no induction of mutations in *Escherichia coli* strain SD-4-73 after exposure to 2-MBT. Neither the doses used nor the source and purity of the 2-MBT were provided by the authors.

Two independent laboratory investigations gave negative results in Ames *S. typhimurium*/microsome assays (40). Strains TA98, TA100, TA1535, and TA1537 were tested with and without S9 activation, at concentrations up to 300 ug 2-MBT/plate. Questionable mutagenic activity was observed only in TA98 cells with S9 activation. The second study detected no mutagenic activity in any of the four strains tested under any conditions.

In mammalian experiments, exposure of V79 (unspecified) cells to doses of 30-500 ug 2-MBT/ml for 4 hours resulted in no increase in 6-thioguanine resistant mutants (39). Results from a Chinese Hamster Ovary (CHO)/HGPRT forward mutation assay conducted with and without activation at doses from 1 to 300 ug/ml were also negative (41). However, mutagenic activity and extreme toxicity were noted at 100 and 150 ug/ml without activation in a mouse lymphoma forward mutation assay (42). Toxicity was reduced with S9 activation, but a significant increase in mutations was noted at high doses (80 and 100 ug/ml).

Cytogenetic assays using 351-451 ug 2-MBT/ml in the presence of rat liver S9 showed increased frequencies of chromosomal aberrations or sister chromatid exchanges (SCEs) in CHO cells. An increased mutation rate was also observed at the TK locus of mouse L5178Y lymphoma cells (14). No significant induction of chromosomal aberrations or SCEs was observed without S9 activation. Although these data indicate that 2-MBT is a clastogen *in vitro*, intraperitoneal injection of 300 mg/kg of 2-MBT did not increase the number of micronucleated polychromatic erythrocytes in the bone marrow of CD-1 mice (41).

Another study was done to evaluate the ability of 2-MBT to increase the incidence of micronucleated polychromatic erythrocytes (PCEs) in bone marrow of

male and female CD-1 mice (61). Two groups of mice (4/sex/group) were given a single intraperitoneal injection of 300 mg 2-MBT/kg and were sacrificed 30 and 48 hours after dosing. Two additional groups (4/sex/group) received two injections of 300 mg/kg at 0 and 24 hours after initial dosing and were sacrificed 48 and 72 hours following initial dosing, respectively. No statistically significant differences between treated and control animals were observed with respect to the number of micronuclei in the PCEs.

A study done to evaluate the genotoxic activity of four rubber accelerators (43) found that 2-MBT disulfide, a structural analog of 2-MBT, did not induce gene reversion in *Salmonella* or *Escherichia coli* WP2 uvrA-, with or without metabolic activation at a dose of 10 ug/ml. Results were also negative for a BALB/3T3 transformation assay in the absence of S9, and for a chromosomal aberration test in cultured CHO cells with or without S9. Exposure of mouse lymphoma cells to 2-MBT disulfide in the absence of activation did not increase the rate of forward mutations at the TK+/- locus. With activation, however, the two highest doses (15 and 30 ug/ml) did produce a significant increase in the rate of forward mutations.

9.0 TERATOGENICITY AND REPRODUCTIVE EFFECTS

Although some studies have been done on teratogenic effects of 2-MBT, results to date are contradictory. In one study, several strains of mice were given up to 464 mg 2-MBT/kg by subcutaneous injection on days 6 through 15 of gestation (44). In two strains, increased incidences of fetal malformations were noted, but only at (unspecified) maternally toxic doses. However, these results were not obtained in a repeat study with one of the strains of mice.

A Russian study (45) found that rats dosed orally with 2-MBT at 20 mg/kg on days 4 and 11 of pregnancy showed a significant increase in embryonic mortality. Criteria for statistical significance and data supporting the decrease in fetal body weight were not reported.

In a more complete study (46), no evidence of teratogenic activity or embryolethality was reported in female rats administered 200 mg 2-MBT/kg intraperitoneally on days 1 through 15 of gestation. Examinations conducted on day 21 of gestation showed no evidence of maternal toxicity, fetal toxicity, or teratogenesis. However, this study is of limited value because it was designed as a screening study and only small group sizes were used.

The teratogenicity of several of the mercaptobenzothiazoles has been evaluated in the chicken embryo (47, 48). 2-MBT at a concentration of 0.10 to 2.0 umol/egg was injected in an acetone vehicle into the heart of 3 day old embryos. Two types of eye defects were frequently found in the malformed embryos, as well as defects of the neck, back, and open coelom. Incidences of specific malformations were not tabulated, although the maximum percentage of malformed embryos was 20%.

In a long term study (37), no cumulative effects on reproduction or lactation were observed in rats fed *ad libitum* 5000 ppm of a formulation containing 2.4% 2-MBT and 27.6% dimethyldithiocarbamate throughout the second generation.

In female rats dosed with at least 400 mg 2-MBT/kg on days 4 to 11 after conception, fetotoxicity and fetal death were observed (63). Further details of the study were not available. Subcutaneous injection of 4176 mg/kg in female mice on days 6 to 14 after conception also resulted in fetotoxicity and specific developmental abnormalities in the eyes, ears, and gastrointestinal tract (64).

10.0 OCCUPATIONAL EXPOSURE

No studies on occupational exposure were located. However, dermal contact and inhalation are expected to be the primary routes of workplace exposure (4). The USEPA states that, as well as worker exposure to 2-MBT during production, exposure is possible during rubber manufacture and reclamation, tire recapping and burning, cleaning of manufacture vessels, drying and grinding of rubber, and handling and emptying of bags used to transport 2-MBT (5). Contact with waste waters from rubber plants is another possible exposure route.

2-MBT is a well-known contact allergen, and several reports of "tennis shoe dermatitis" are available (49-53). Allergic dermatitis has been traced to 2-MBT incorporated into clothing articles and shoes. Several products under Food and Drug Administration jurisdiction in the United States have also been shown to contain residual 2-MBT. The chemical has been shown to leach out of numerous commercial products, including the stoppers of 500 ml infusion bottles, single-dose injection syringes, rubber baby bottle nipples, rubber pharmaceutical products, and rubber articles which contact food (5).

Human exposure to 2-MBT in ambient air has been estimated using an air dispersion model, and from modeling using 2,2'-dithiobis(benzothiazole) (MBTS) (5). Air concentrations of MBTS within 1000 metres of an elastomer manufacturing site were estimated at 0.01 mg/m^3, stated in the source to be equivalent to an inhalation exposure of 0.035 mg/kg/day. This estimated value probably approximates that of 2-MBT.

11.0 ANALYTICAL METHODS OF DETECTION

The Southern Research Institute used high pressure liquid chromatography (HPLC) to monitor the purity of radiolabelled 2-MBT used in pharmacokinetic studies in mice and rats (16, 17, 18). Gradient elution with 20 mM acetic acid in 40% and 85% aqueous acetonitrile was used with radioactivity monitoring and UV absorbance at 254 nm. The more polar metabolites of 2-MBT in the urine were similarly analyzed except a combination of isocratic and gradient elution of 20 mM phosphoric acid in 20, 30, or 40% aqueous acetonitrile

was also used. The detection limits of the method were not indicated in the available summaries.

A Japanese group (54) was able to achieve detection limits of 1.0 ug/g for fish tissues, and 10 ppb for water samples through extraction with methyl isobutylketone and analysis by HPLC.

Another Japanese group measured 2-MBT in water and sediment by extracting samples with methylene chloride and analyzing the extracts by gas-liquid chromatography using a flame photometric detector. Detection limits of 40 ppb for water and 2 ppb for sediment were achieved (55).

A USEPA sponsored study of the determination of 2-MBT in waste waters reported that recoveries of 79% at the 5 ppb level were possible following extraction of samples with methylene chloride, extract partitioning on silica gel, and analysis by HPLC with UV detection (56).

Finally, Environment Canada has recently developed a liquid chromatography method for determining 2-MBT levels in effluents and sediments (19). The sample is extracted with methylene chloride, filtered, and concentrated. The residue is dissolved in acetonitrile and the sample is then analyzed for 2-MBT by HPLC. Detection limits are 25 ppb.

12.0 EXISTING LEGISLATION

Legislation on allowable discharge or ambient levels of 2-MBT for British Columbia were not located. However, the American Conference of Governmental Industrial Hygienists (ACGIH) has established an eight hour threshold limit value (TLV) of 15 mg/m^3 for total dust, and a respirable TLV of 5 mg/m^3 (4).

13.0 CONCLUSION

The effects of 2-MBT in the environment may be most pronounced in aquatic systems, since significant amounts may enter water in effluents, or in groundwater contaminated with 2-MBT dust. Since 2-MBT is toxic to several fish species, it is important to consider regulating release of the compound to the environment. In British Columbia, there appears to be a lack of appropriate regulations relating to environmental release of the chemical, especially in the lumber industry. Runoff from lumber yards has been studied, but more studies are necessary to determine wood treatment procedures which would minimize aquatic impacts of 2-MBT from this source.

Similarly, the environmental and toxic impacts of degradation products of 2-MBT should be further characterized. The methylation of 2-MBT to MTBT, a more bioaccumulable compound, is of some concern, since this compound could have more severe toxicological consequences than the parent compound. Further

freshwater and marine acute and chronic toxicity testing should also be done to characterize the toxicology of 2-MBT in the aquatic environment.

The risk of 2-MBT to human health appears to be minimal, the only substantiated effect being several reported cases of contact dermatitis. However, contradictory results from carcinogenicity, mutagenicity, and teratogenicity studies indicate that further testing is necessary.

Because comparative studies of 2-MBT sources in the environment are not available, it is difficult to assess the relative ecological risks associated with the use of TCMTB in the lumber industry. Since 2-MBT is the main breakdown product of TCMTB, and some mills in Canada use TCMTB antisapstain formulations, contribution from mills may be significant. However, the decline in the use of TCMTB as an antisapstain agent would mean a decline in 2-MBT from this source. Also, because several other vulcanizing agents, besides 2-MBT, are used in the rubber industry, the environmental impact of 2-MBT from lumber mill sources could be relatively low when compared to this potential source. Further studies in these areas should be done to allow assessment of relative impacts from different sources.

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TABLE 1: A Comparison of Toxicity Values for 2-MBT and TCMTB

	Oral LD50* Rat - mg/kg	Dermal LD50* Rabbit - mg/kg	Aquatic 96hrLC50 Fish species**-ppm
TCMTB	1200[1] (80% form)	>2000[1] (80% form)	0.039[1] Chinook Salmon 0.015[1]
Busan 1030***	1200[1]	1080[1]	0.117[1]
2-MBT	500[10]	>5010[11]	0.73[32]

*Values quoted are the lowest published for each species in fresh water. TCMTB has been shown to be more acutely toxic to fish in sea water (1).

**Fish species is Rainbow trout unless otherwise noted.

***Busan 1030 is a solvent based 30% TCMTB formulation used in wood treatment.

APPENDIX A

INFORMATION SOURCES SEARCHED FOR CITATIONS ON 2-MBT

The following computerized databases were searched for information on 2-MBT:

CA Search
Toxline
Toxlit
AQUIRE
Medline
RTECS
CCOHS

APPENDIX B

MAMMALIAN LETHAL TOXICITY CLASSES FOR CHEMICAL COMPOUNDS*

Toxicity Rating	Commonly Used Term	Oral/Dermal** (mg/kg)	Inhalation‡ (ppm-mg/L)
1	Practically Nontoxic	>15,000	>200
2	Slightly Toxic	5,000-15,000	20-200
3	Moderately Toxic	500-5,000	2-20
4	Very Toxic	50-500	0.2-2
5	Extremely Toxic	5-50	0.02-0.2
6	Ultra Toxic	<5	<0.02

*Toxicity ratings adapted from references 10, 70, and 72.

**Index: acute oral or dermal LD50.

‡Index: LC50 - four hour inhalation exposure.

APPENDIX C

AQUATIC LETHAL TOXICITY CLASSES FOR CHEMICAL COMPOUNDS*

Toxicity Rating	Commonly Used Term	Exposure Conc.** (ppm-mg/L)
1	Practically Nontoxic	>100
2	Slightly Toxic	10 to 100
3	Moderately Toxic	1 to 10
4	Very Toxic	0.1 to 1
5	Extremely Toxic	0.01 to 0.1
6	Ultra Toxic	<0.01

*Toxicity ratings adapted in consultation with reference 71.

**Index: 96 hour LC50 or EC50.